National Institute of Standards & Technology

Report of Investigation

Reference Material 8415

Whole Egg Powder

A Joint Material of Agriculture Canada and NIST

Distributed by the National Institute of Standards and Technology

Reference Material (RM) 8415 is intended for use in evaluating analytical methods and instruments used for the determination of major, minor, and trace constituent elements, as well as proximates, fatty acids, and calories in egg, egg products, and similar food, agricultural, and biological materials. This material can also be used for quality assurance when assigning values to in-house control materials. RM 8415 consists of 35 g of dry whole egg powder packaged in a glass bottle sealed in an aluminum-nylon pouch.

Reference Concentration Values: Reference concentration values for major, minor, and trace constituent elements are provided in Table 1. Reference concentration values for proximates, calories, and fatty acids are provided in Table 2. The reference values in Tables 1 and 2 were derived from results reported in an interlaboratory comparison exercise and by four additional collaborating laboratories, respectively. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Information Concentration Values: Information concentration values for additional elements, fatty acids, and water-soluble vitamins are provided in Tables 3, 4, and 5. These are noncertified values with no reported uncertainties as there is insufficient information to assess uncertainties. The information values are given to provide additional characterization of the material. Use of this RM to quantitatively monitor method performance for analytes other than those with reference concentration values in Tables 1 and 2 is not recommended.

Expiration of Value Assignment: The value assignment of this RM lot is valid until **24 February 2008**, within the measurement uncertainties specified, provided the RM is handled and stored in accordance with the instructions given in this report. Value assignment is nullified if the RM is damaged, contaminated, or modified.

Maintenance of RM Value Assignment: NIST will monitor this RM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

Statistical support was provided by M.S. Wolynetz, Statistical Research Section, Research Program Service, Agriculture Canada and L.M. Gill, Statistical Engineering Division, NIST.

Support aspects involved with the value assignment and issuance of this RM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and W.R. Wolf (U.S. Department of Agriculture).

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Revised Report Issue Date: 28 April 1999

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See Report Revision History on Last Page

RM 8415 was prepared at Agriculture Canada under the direction of M. Ihnat, Centre for Land and Biological Resources Research (CLBRR). Coordination of the technical measurements leading to the value assignment of this RM was performed by M. Ihnat of CLBRR, Agriculture Canada and K.E. Sharpless and S.A. Wise of the NIST

Analytical Chemistry Division. Following the original analyses for elemental value assignment by the laboratories listed in Appendix A, the material was distributed by NIST to Covance Laboratories (Madison, WI), Lancaster Laboratories (Lancaster, PA), Medallion Laboratories (Minneapolis, MN), and Southern Testing and Research Laboratories (Wilson, NC) for the measurement of proximates, calories, and fatty acids. RM 8415 was also distributed in an interlaboratory comparison exercise in 1995; information values for the concentrations of several water-soluble vitamins have been assigned based on results reported by the laboratories listed in Appendix C.

NOTICE AND WARNING TO USERS

Storage: Until required for use, RM 8415 should be stored at room temperature in its original bottle, tightly-capped and not exposed to intense direct light or ultraviolet radiation.

Warning: For laboratory use only. Not for human consumption.

Instructions for Use: Prior to each use, contents of the bottle should be well mixed by gentle shaking and rolling of the container. A minimum subsample size of 0.5 g should be taken for analysis. Moisture content should be determined on a separate subsample for conversion of analytical results to a dry-mass basis. The recommended method of drying to relate analytical results to the reference values listed in the tables is drying for 4 h in an air oven at 85 °C. Dissolution procedures for elemental analyses should be capable of rendering a completely dissolved sample appropriate to the method and should be designed to avoid losses of elements by volatilization or by retention on decomposition and processing containers and measuring equipment. Analytical methods should be capable of measuring total levels of elements for comparison with reference values.

PREPARATION AND ANALYSIS

Preparation: The source of material for RM 8415 was Canada grade A dried whole egg powder, containing added color and a maximum of 2 % Zeolex (sodium silico-aluminate anti-caking ingredient) obtained from Vanderpol's Eggs Ltd., Surrey, BC, Canada. All preparatory work following acquisition of the commercial product was performed at the facilities of Agriculture Canada, Ottawa, Ontario, Canada [1,2]. The dry bulk powder was sterilized with cobalt-60 gamma radiation to 2.0 megarads by Atomic Energy of Canada Ltd. Material sieving was through nylon monofilament sieve cloths supported in high-density white polyethylene holders. Pairs of sieves with openings of approximately 250 μm and 50 μm were used to yield a middle-cut fraction for use as the reference material. This fraction was blended in a polymethylmethacrylate V-configuration blender and packaged into clean 150 mL brim capacity, colorless glass bottles with triseal (polyethylene)-lined white polypropylene screw caps. A total of 144 randomly selected units were used for physical and chemical characterization in the original analyses. Units were individually hermetically sealed in aluminum-nylon pouches to enhance long-term stability.

Homogeneity Assessment: Homogeneity testing was performed on randomly selected units for nine elements in three laboratories [3,4]. Subsamples of 0.5 g and 2.0 g were taken from a total of four units and analyzed by M. Ihnat, Agriculture Canada, for aluminum, calcium, iron, potassium, magnesium, strontium, and zinc using acid digestion flame atomic absorption spectrometry [4-7]. Subsamples of 1.0 g to 2.0 g each, taken from a total of six units, were analyzed by R.W. Dabeka, Health and Welfare Canada, for lead by graphite furnace atomic absorption spectrometry following acid digestion and separation and preconcentration of the analyte using coprecipitation with palladium and ascorbic acid [8]. Solid sampling graphite furnace atomic absorption spectrometric determinations were performed by M. Stoeppler and U. Bagschik, Nuclear Research Center, Jülich, Federal Republic of Germany, on a total of 40 subsamples of 0.5 mg each, from a total of four units for copper [2]. In addition, the analytical results obtained from a large number of analysts (Appendix A) participating in an interlaboratory comparison exercise were assessed to provide homogeneity estimates for other elements [2,4]. No statistically significant heterogeneity was found for aluminum, calcium, iron, lead, magnesium, manganese, mercury, phosphorus, potassium, selenium, sodium, strontium, and zinc in sample sizes required by the analytical technique ranging from 0.1 g to 2 g. Data for all analytes (including the proximates and fatty acids) have been statistically treated as though they are homogeneous, although the homogeneity of other analytes has not been investigated.

Value Assignment: Chemical analyses to establish reference concentrations of elements were conducted in an interlaboratory comparison exercise involving Agriculture Canada and selected analysts in other laboratories (Appendix A) using analytical methods listed in Table 6. Analyses were performed by each participant on duplicate subsamples from randomly selected (typically four) units of material; subsample sizes and methods were left to the discretion of the analyst. Subsample sizes ranged from 0.001 g to 5 g, typically 0.4 g. Elemental determinations were performed on the

material "as received," with conversion of results to a dry-mass basis using moisture values determined on separate 2 g subsamples by the drying procedure specified in the "Instruction for Use" section of this report.

Following the original elemental determinations, NIST distributed RM 8415 to four laboratories (Appendix B) for measurement of proximates, fatty acids, and calories. Each laboratory analyzed one portion from each of three bottles of RM 8415 using their routine methods (Table 7). Determinations were performed on the material "as received," with conversion of results to a dry-mass basis using moisture values determined on separate subsamples taken from each of the three bottles. Standard Reference Material (SRM) 1846 Infant Formula was analyzed for quality assurance. RM 8415 was also analyzed by several laboratories participating in an interlaboratory comparison exercise in 1995; several of these laboratories (Appendix C) reported values for water-soluble vitamins, and these results are provided as information values in Table 5.

Table 1. Reference Concentrations of Constituent Elements

Major Constituents

Element	Mass Fraction (%)[a]	Methods[b]
Nitrogen[c]	6.30 ± 0.13	I01, J01, J02
Phosphorus	1.001 ± 0.032	B02, B03, F01, F02, M01
Sulfur	0.512 ± 0.050	B02, D04, J04, M02
Chlorine	0.508 ± 0.032	D01, D04, K02
Sodium	0.377 ± 0.034	A03, B01, B02, B03, D01
Potassium	0.319 ± 0.037	A01, A03, B02, B03, B04, D04
Calcium	0.248 ± 0.019	A01, B02, B04, D01

Minor and Trace Constituents

Element	Mass Fraction (mg/kg)[a]	Methods[b]
Aluminum	540 ± 86	A05, B02, B03, B04, D01
Magnesium	305 ± 27	A01, A03, B02, B03, B04, D01
Iron	112 ± 16	A01, A03, B02, B03, B04, D02, D03
Zinc	67.5 ± 7.6	A01, A03, B02, B03, D02, D03, H01
Strontium	5.63 ± 0.46	A01, B02, B03
Copper	2.70 ± 0.35	A05, A06, B02, C06, D03, H01
Iodine	1.97 ± 0.46	D03, D05, F01, H05
Manganese	1.78 ± 0.38	A01, A03, A05, B02, B04, D01, D03
Selenium	1.39 ± 0.17	A08, C01, C04, D01, D02, D03, G01
Vanadium	0.459 ± 0.081	B02, D01, D03
Boron	0.41 ± 0.26	B02, C09, D04
Chromium	0.37 ± 0.18	A05, A06, A12, B02, C05, D02, D03
Molybdenum	0.247 ± 0.023	C06, D03, H06
Lead	0.061 ± 0.012	A16, H01
Cobalt	0.012 ± 0.005	D01, D02, D03, H01
Mercury	0.004 ± 0.003	A09, A10, D03

[a] Reference values, expressed as mass fractions, are based on the dry material, dried according to instructions in this report, and are equally weighted means of results from at least two, but typically several, different analytical methods applied by analysts in different laboratories. Uncertainties are imprecision estimates expressed either as a 95 % confidence interval or occasionally (B, Co, K, Mn, Mo, Zn) as an interval based on the entire range of accepted results for a single future determination, based on a sample mass of at least 0.5 g. These uncertainties, based on among-method, among-laboratory, among-unit, and within-unit estimates of variances, include measures of analytical method and laboratory imprecisions and biases. (NIST has replaced the previously used term "best estimate" with "reference value.")

[b] Analytical method codes and descriptions are provided in Table 6.

[c] Nitrogen results have been updated to include results from four additional collaborating laboratories (Appendix B). Only the uncertainty has changed from that provided with the original assigned value.

Table 2. Reference Concentrations of Proximates, Selected Fatty Acids (as Triglycerides), and Calories

Analyte	Mass Fraction, as received (%)[a,b]	Mass Fraction, dry-mass basis (%)[a]
Moisture	3.53 ± 0.54	0 (by definition)
Solids	96.47 ± 0.54	100 (by definition)
Ash	4.78 ± 0.53	4.96 ± 0.55
Protein[c]	37.8 ± 1.2	39.2 ± 1.1
Carbohydrate	7.5 ± 5.1	7.8 ± 5.3
Fat	46.4 ± 4.9	48.0 ± 5.0
Tetradecanoic Acid (C14:0) (Myristic Acid)	0.184 ± 0.012	0.191 ± 0.013
Pentadecanoic Acid (C15:0)	0.048 ± 0.004	0.050 ± 0.003
Hexadecenoic Acid (C16:0) (Palmitic Acid)	11.92 ± 0.38	12.36 ± 0.45
9-Hexadecenoic Acid (C16:1) (Palmitoleic Acid)	1.54 ± 0.35	1.59 ± 0.37
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.151 ± 0.027	0.156 ± 0.029
Octadecanoic Acid (C18:0) (Stearic Acid)	4.06 ± 0.23	4.21 ± 0.26
(Z)-9-Octadecenoic Acid (C18:1) (Oleic Acid)	22.2 ± 2.3	23.0 ± 2.4
(Z,Z)-9,12-Octadecadienoic Acid (C18:2) (Linoleic Acid)	4.52 ± 0.35	4.69 ± 0.38
γ-Eicosadienoic Acid (C20:3)	0.062 ± 0.006	0.064 ± 0.007
5,8,11,14-Eicosatetraenoic Acid (C20:4) (Arachidonic Acid)	0.478 ± 0.042	0.496 ± 0.045
Docosahexaenoic Acid (C22:6)	0.257 ± 0.061	0.266 ± 0.062
Calories[c]	(598 ± 24) kcal/100 g	(620 ± 23) kcal/100 g

[a] Each reference concentration value, expressed as a mass fraction on an as-received or dry-mass basis, is an equally weighted mean of results from the laboratories shown in Appendix B. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the *ISO Guide to the Expression of Uncertainty in Measurement* [9]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Table 7.

[b] The "as received" values are based on the moisture content at the time the measurements for value assignment were performed. The amount of moisture in this material may change if moisture is transferred to or absorbed from the atmosphere during storage.

[c] The protein concentration was calculated from the nitrogen values reported by the laboratories shown in Appendix B using a conversion factor of 6.25; subsequent calculations of carbohydrates and calories were also based on these protein concentrations. The nitrogen values reported by the laboratories shown in Appendix B were combined with the original data for calculation of the reference value for nitrogen provided in Table 1.

[d] The value for calories is the mean of the individual caloric calculations. If the mean proximate values are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 598 kcal/100 g and 620 kcal/100 g on an as-received and dry-mass basis, respectively.

Table 3. Information Concentrations of Constituent Elements

Element	Mass Fraction (mg/kg)[a]	Methods[b]
Antimony	0.002	D02, D03
Arsenic	0.01	D03
Barium	3	B02, B03, B04
Cadmium	0.005	A06, D03, H01

[a] These analytical values, on a dry-mass basis, are estimates given strictly for information only, as they are based on results of a limited number of determinations or from only one method; no uncertainties are provided.
[b] Analytical method codes and descriptions are provided in Table 6.

Table 4. Information Concentrations of Selected Fatty Acids (as Triglycerides)

Analyte	Mass Fraction, as received (%)[a]	Mass Fraction, dry-mass basis (%)[a]
Dodecanoic Acid (C12:0) (Lauric Acid)	0.0055	0.0057
9-Tetradecenoic Acid (C14:1) (Myristoleic Acid)	0.048	0.049
9-Octadecenoic Acid (C18:1) (Z-Elaidic Acid)	0.33	0.34
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3) (Linolenic Acid)	0.27	0.28
ϒ-Linolenic Acid (C18:3)	0.029	0.030
9-Eicosensic Acid (C20:1) (Gadoleic Acid)	0.14	0.15
Docosapentaenoic Acid (C22:5)	0.037	0.038

[a] These information values, reported on an as-received or dry-mass basis, are the equally weighted means of results reported by the laboratories shown in Appendix B. These values are based on results from determinations by two or three of the laboratories and are included to provide additional characterization of the material; no uncertainties are provided. Analytical methodology information is provided in Table 7.

Table 5. Information Concentrations of Selected Vitamins

Analyte	Mass Fraction, as received (mg/kg)[a]	Mass Fraction, dry-mass basis (mg/kg)[a]
Vitamin B_1	2.8	2.9
Vitamin B_2	12	13
Vitamin B_6	3.7	3.8
Vitamin B_{12}	0.068	0.070
Biotin	1.3	1.3
Folic Acid	1.8	1.8
Inositol	860	890
Niacin	2.4	2.5
Pantothenic Acid	91	94

[a] These information values, reported on an as-received or dry-mass basis, are the equally weighted means of results reported by the laboratories shown in Appendix C. These values are based on results from determinations by one to four laboratories, and are included to provide additional characterization of the material; no uncertainties are provided. Analytical methodology information is provided in Table 8.

Table 6. Analytical Methods Used by Collaborating Laboratories (Appendix A) to Determine Reference and Information Concentration Values of Elements[a]

Analytical Method	Code	Elements Determined
Acid digestion flame atomic absorption spectrometry	A01	Ca, Fe, K, Mg, Mn, Sr, Zn
Dry ashing flame atomic absorption spectrometry	A03	Fe, K, Mg, Mn, Na, Zn

Closed vessel acid digestion electrothermal atomic absorption spectrometry	A05	Al, Cr, Cu, Mn
Dry ashing electrothermal atomic absorption spectrometry	A06	(Cd), Cr, Cu
Dry ashing hydride generation atomic absorption spectrometry	A08	Se
Acid digestion cold vapor atomic absorption spectrometry	A09	Hg
Closed vessel acid digestion cold vapor atomic absorption spectrometry with preconcentration	A10	Hg
Dry ashing digestion electro-thermal atomic absorption spectrometry	A12	Cr
Acid digestion coprecipitation electrothermal atomic absorption spectrometry	A16	Pb
Acid digestion atomic emission spectrometry	B01	Na
Acid digestion inductively coupled plasma atomic emission spectrometry	B02	Al, B, (Ba), Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Sr, V, Zn
Closed vessel acid digestion inductively coupled plasma atomic emission spectrometry	B03	Al, (Ba), Fe, K, Mg, Na, P, Sr, Zn
Dry ashing inductively coupled plasma atomic emission spectrometry	B04	Al, (Ba), Ca, Fe, K, Mg, Mn,
Acid digestion isotope dilution mass spectrometry	C01	Se
Acid digestion dry ashing hydride generation isotope dilution inductively coupled plasma mass spectrometry	C04	Se
Dry ashing acid digestion isotope dilution mass spectrometry	C05	Cr
Acid digestion isotope dilution inductively coupled plasma mass spectrometry	C06	Cu, Mo
Neutron activation mass spectrometry	C09	B
Instrumental neutron activation analysis	D01	Al, Ca, Cl, Co, Mg, Mn, Na, Se, V

Instrumental neutron activation analysis with acid digestion	D02	Co, Cr, Fe, (Sb), Se, Zn
Neutron activation analysis with radiochemical separation	D03	(As), (Cd), Co, Cr, Cu, Fe, Hg, I, Mn, Mo, (Sb), Se, V, Zn
Neutron capture prompt gamma activation analysis	D04	B, Cl, K, S
Epithermal instrumental neutron activation analysis	D05	I
Acid digestion light absorption spectrometry	F01	I, P
Dry ashing light absorption spectrometry	F02	P
Acid digestion fluorometry	G01	Se
Closed vessel acid digestion anodic stripping voltammetry	H01	(Cd), Co, Cu, Pb, Zn
Acid digestion differential pulse polarography	H05	I
Dry ashing catalytic adsorption polarography	H06	Mo
Kjeldahl method for nitrogen - volumetry	I01	N[b]
Combustion elemental analysis - thermal conductivity	J01	N[b]
Combustion elemental analysis with chromatographic separation - thermal conductivity	J02	N[b]
Combustion elemental analysis - fluorometry	J04	S
Dry ashing volumetry	K02	Cl
Acid digestion gravimetry	M01	P
Dry ashing gravimetry	M02	S

[a] Letter codes refer to classes of similar methods; number codes refer to specific variants. Elements in parentheses have only information values in this RM. (NIST has replaced the previously used term "best estimate" with "reference value.")

[b] See Table 7 for additional information.

Table 7. Methods Used by Collaborating Laboratories (Appendix B) for the Determination of Proximates, Calories, and Fatty Acids

Ash	mass loss after ignition in a muffle furnace
Calories	calculated; [(9 × fat)+ (4 × protein) + (4 × carbohydrate]
Carbohydrate	calculated; [solids – (protein + fat + ash)]
Fat	sum of individual fatty acids
Fatty acids	hydrolysis followed by gas chromatography

Moisture	mass loss after drying in a vacuum oven (3 laboratories); mass loss after drying in a forced-air oven (1 laboratory)
Nitrogen	Dumas (1 laboratory); modified Dumas (1 laboratory); Kjeldahl (2 laboratories). Note that in the original elemental determinations 12 laboratories provided results (one laboratory provided results using two techniques): Kjeldahl (7); combustion - thermal conductivity (4), and combustion - chromatographic separation - thermal conductivity (2).
Protein	calculated from nitrogen using a factor of 6.25
Solids	calculated; (sample weight – moisture)

Table 8. Methods Used by Collaborating Laboratories (Appendix C) for the Determination of Vitamins

Vitamin B_1	microbiological (1 laboratory); digestion with fluorescence detection (3 laboratories)
Vitamin B_2	microbiological (1 laboratory); digestion with fluorescence detection (2 laboratories)
Vitamin B_6	microbiological (2 laboratories)
Vitamin B_{12}	microbiological (2 laboratories)
Biotin	microbiological (2 laboratories)
Folic Acid	microbiological (3 laboratories)
Inositol	microbiological (1 laboratory)
Niacin	microbiological (1 laboratory); acid digestion with absorption spectrophotometry (1 laboratory)
Pantothenic Acid	microbiological (2 laboratories)

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Appendix A. Collaborating Analysts for Elemental Determinations

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Appendix B. Collaborating Laboratories for Proximate, Fatty Acid, and Caloric Determinations

Covance Laboratories, Madison, WI, USA.
Lancaster Laboratories, Lancaster, PA, USA.
Medallion Laboratories, Minneappolis, MN, USA.
Southern Testing and Research Laboratories, Wilson, NC, USA.

Appendix C. Collaborating Laboratories for Water-Soluble Vitamin Determinations

Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC, USA.
Covance Laboratories, Madison, WI, USA.
Lancaster Laboratories, Lancaster, PA, USA.
Southern Testing and Research Laboratories, Wilson, NC, USA.

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